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Determination of metals in different tissues of *Trachurus trachurus* from the Marmara Sea and evaluation of their health risks

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ABSTRACT

The present study aimed to determine the differences in metal levels of Aluminum (Al), Cadmium (Cd), Chromium (Cr), Copper (Cu), İron (Fe), Manganese (Mn), Nickel (Ni), Lead (Pb) and Zinc (Zn) between muscle and liver tissues of Trachurus trachurus, the correlation of metal accumulation in tissues with fish length and weight was examined. Health risks and nutritional adequacy were evaluated based on metal levels accumulated in fish muscle. The results demonstrated that fish liver accumulated higher metal levels than muscle. The investigation revealed that the majority of metals present in the tissues of the fish did not exhibit a significant correlation with their size ($p>0.05$). It can be concluded that fish are not an adequate source of these nutrients because their nutrient levels are below reference values. The fish were found to be nutrient deficient, as the levels of nutrients were below reference values. The estimated daily intakes of all metals accumulated in muscle were below reference doses. The target hazard coefficient and target hazard index values were less than 1. Lifetime carcinogenic risk values were below 10^{-4}, and total carcinogenic risk values were within the acceptable range. Pb and Cd concentrations in muscle exceeded the tolerable limit. The daily amount of fish that could be consumed without non-carcinogenic health risk was 181 grams for Cd and 295 grams for Pb. The daily amount of fish that could be consumed without carcinogenic health risk was 1.21 grams for Cd and 231 grams for Pb.

Keywords: *Trachurus trachurus*, Heavy metal, Risk assessment, Marmara Sea, Carcinogenic risk

Introduction

Many anthropogenic sources, including industrial, agricultural, domestic, and mineral wastes contaminate marine ecosystems. The presence of heavy metals in the sea can result in the exposure of humans and other organisms to these metals through a multitude of pathways. The primary pathway for human exposure to heavy metals found in the marine environment is consuming seafood. Since fish is the most consumed seafood product by humans, examining the accumulation of heavy metals in fish species is crucial.

Some heavy metals can exert a deleterious effect on organisms, while certain macroelements are essential for maintaining optimal health. Non-essential heavy metals, such as Al, Cd and Pb, can exhibit toxic effects even when consumed in low concentrations. (Cardoso et al., 2019). Heavy metals, including Cr, Cu, Fe, Mn, Ni and Zn, are essential elements. However, exposure to them in excessive concentrations can lead to a range of adverse health effects, including hepatic, renal, cardiovascular and neurological disorders. (Korkmaz et al., 2017).

Heavy metal pollution of the sea results in the accumulation of heavy metals in marine organisms. Living organisms cannot metabolise these pollutants, as they are stable, do not biodegrade, and therefore cannot accumulate. (Lozano-Bilbao et al., 2020). Organs such as the liver, gonads, kidneys, gills, and skin are target organs that accumulate metal in aquatic organisms. These toxic metals, which are absorbed into solids dissolved or undissolved in seawater, are absorbed by aquatic organisms through the gills and skin (Jabeen et al., 2018). Metal concentrations detected in the gills reflect the metal concentrations in the water, while concentrations in the liver indicate the accumulation of metals in vivo (Kalantzi et al., 2019; Tokatli, 2018; Yilmaz, 2003). It is well established that muscle tissue is not active in metal accumulation (Sunlu et al., 2001). Conversely, research has indicated that metal accumulation in skin tissues is more prevalent than in muscle tissues (Yazkan et al., 2002; Yilmaz, 2003).

Atlantic horse mackerel, Trachurus trachurus (Linnaeus, 1758), is a pelagic fish of significant economic importance (Bektas & Belduz, 2009; Turan et al., 2009). It is stated that this fish species is rich in minerals, vitamins and polyunsaturated fatty acids (Ozden, 2010). These fish species are common in the Aegean, Mediterranean and Black Sea, including mostly in the heavily polluted Marmara Sea (Erkan et al., 2020; Turan et al., 2009).

The Marmara Sea is influenced by many pressures, such as urban and industrial wastewater, maritime traffic, agricultural and settlement activities on land, and tourism. Recent studies have shown that the Marmara Sea remains polluted, particularly due to the increase in heavy metal, microplastic, and radionuclide concentrations (Almas et al., 2022; Baysal & Saygin, 2022; Gözel et al., 2022; Tan, 2021). Fishing activities continue to be conducted at a high intensity in this region. Consequently, it is necessary to monitor the metal content of seafood caught in this sea to know whether it suits human consumption. There are few studies on the content of metals and trace elements in *Trachurus trachurus* in the Marmara Sea region (Aydın & Tokalıoğlu, 2015; Mutlu et al., 2012; Yaman et al., 2013). Additionally, few risk assessments have been conducted for fish species cultivated in the Sea of Marmara. However, no carcinogenic or non-carcinogenic risk assessment exists for consuming fish from the Trachurus trachurus species. Humans may also consume liver, stomach, and muscle tissues when they consume fish. Fishermen can also recycle these tissues to feed the fish (Onsanit et al., 2010). For this purpose, the concentrations of Al, Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn were determined and their relationship with biometric data was analysed in the tissues (muscle and liver) of *Trachurus trachurus* species grown in the Marmara Sea. In addition, for people consuming this fish, the adequacy of nutrients was evaluated, and carcinogenic-noncarcinogenic risk assessment was carried out in muscle tissues.

Materials and Methods

Sample Collection and Preparation

Ten fish samples of Trachurus trachurus were randomly collected from local fishermen in Istanbul who fish in different parts of the Sea of Marmara. Sampling was conducted in December. No distinction was made between the sexes during sampling. The laboratory received the fish samples on the same day. The fish's length and weight were measured, and then samples were washed with distilled water for analysis. Each fish's muscle and liver tissue were dissected using a plastic knife. The tissues were homogenised into small pieces. Three fish muscle and liver tissue replicates were prepared and stored at -20°C until analysis.

Sample Digestion and Metal Analysis

The method employed by (Dirican et al., 2015) for the digestion of fish samples was utilised in this study. Weighed 1 g wet samples of muscle and liver tissues and placed in glass vials. 3 mL concentrated nitric acid was added to the samples and incubated at room temperature for 24 hours. Then, 1 mL of sulfuric acid and 1-2 drops of nitric acid were added and heated on a hot plate. Heating was continued until the dissolution of the samples was complete, and a transparent solution was obtained. The digested samples were diluted to 25 mL with distilled water. Sample solutions containing particles were filtered through Whatman No. 2 filter paper and ready for analysis. Flame Atomic Absorption Spectrometer - FAAS (Agilent 240 Duo) was calibrated with a standard solution of metals, and the method's accuracy was calculated using the standard addition method. The measurement parameters by FAAS are given in Table 1. The fish samples' Al, Cd, Cr, Cu, Fe, Ni, Mn, Pb and Zn metal concentrations were then measured three times with the spectrometer. The results were statistically evaluated, and carcinogenic and noncarcinogenic risk assessment was performed.

Risk Assessments

Estimated daily heavy metal intake of Trachurus trachurus

The estimated daily intake (EDI) is the amount of a substance that can be consumed daily based on body weight. The estimated daily heavy metal intake of *Trachurus trachurus* was calculated by using the equation below (Storelli et al., 2020; Uroko et al., 2020).

$$EDI = \frac{CM \times DIF}{BW} \qquad (1)$$

Where;

CM = Concentration of metal (mg/kg). The mean metal concentration determined in the muscle tissue of fish was used.

DIF = Daily intake of fish (g/day). The amount of fish portion consumed daily in Turkiye (18.4 g/day) was used (GDFA, 2021).

BW = Body weight. The average body weight of fish consumers was 70 kg for an adult.

Target hazard quotient

The target hazard quotient (THQ) was calculated to assess the non-carcinogenic health risks of heavy metals. Trachurus trachurus was consumed. THQ values were calculated for each heavy metal using the following equation (Li et al., 2021).

$$THQ = \frac{EF \times ED \times DIF \times CM}{RfDo \times BW \times AT_{nc}} \times 10^{-3} \qquad (2)$$

Where;

EF = exposure frequency (365 days/year) (USEPA, 1991).

ED = exposure duration (26 years) (USEPA, 2022a).

RfDo = chronic oral reference dose (mg/kg/day).

AT_{nc} = average exposure time to non-carcinogens (365 days/year × ED) (USEPA, 2022c).

If the THQ values are below 1, there is no negative effect on human health. If they are above 1, consumers may experience a negative health situation.

Target hazard index

The hazard index (THI) equation was used to determine the potential risk triggered by ingesting more than one heavy metal. The total risk calculation was made by taking the sum of the THQ values calculated for each metal. The hazard index percentage for each metal was also calculated. (USEPA, 1989).

$$HI = \sum_{n}^{i} THQ_{n\,Metal} \qquad (3)$$

$$\%\, HI_n = \frac{THQ_n}{HI} \times 100 \qquad (4)$$

Table 1. Measurement parameters for determination of the metals by FAAS.

	Al	Cd	Cr	Cu	Fe	Ni	Mn	Pb	Zn
Wavelength (nm)	309.3	326.1	357.9	324.8	372.0	352.5	279.5	283.3	213.9
Lamp Current (mA)	10.0	4.0	7.0	4.0	5.0	4.0	5.0	10.0	5.0
Slit Width (nm)	0.5	0.5	0.2	0.2	0.2	0.2	0.2	0.5	1.0
Air Flow (mL/min)	-	13.5	-	13.5	13.5	13.5	13.5	13.5	13.5
Acetylene Flow (mL/min)	6.8	2.0	6.8	2.0	2.0	2.0	2.0	2.0	2.0
N_2O Flow (mL/min)	10.0	-	10.0	-	-	-	-	-	-

Lifetime carcinogen risk

The lifetime carcinogen risk (LCR) refers to the increased likelihood of developing cancer during a person's lifetime due to exposure to a carcinogen. The lifetime cancer risk from consuming *Trachurus trachurus* fish was estimated using the following equation. (USEPA, 2022a; Varol et al., 2022).

$$LCR = \frac{EF \times ED \times DIF \times CM \times CSFo}{BW \times AT_{ca}} \times 10^{-3} \quad (5)$$

Where;

CSFo = oral cancer slope factor of the metals (mg/kg/day).

AT_{ca}= average exposure time to carcinogens (365 days/ year × LT) (USEPA, 2022c).

LT= Lifetime. Turkiye's average life expectancy was 78 years. (TUİK, 2018).

Total cancer risks

The total cancer risks (TCR) due to exposure to multiple carcinogenic heavy metals through the consumption of *Trachurus trachurus* fish was calculated as the sum of the cancer-producing risks of each heavy metal. (Liu et al., 2013).

$$TCR = \sum_k^i LCR_k \quad (6)$$

Safe consumption limits

The safe consumption limits (SCL) were calculated to determine the amount of fish that can be safely consumed for a given period with respect to non-carcinogenic and carcinogenic health effects. (USEPA, 2000; Varol et al., 2017).

$$SCL_{nc} = \frac{RfDo \times BW}{CM} \quad (7)$$

$$SCL_{ca} = \frac{ARL \times BW}{CM \times CSFo} \quad (8)$$

Where;

SCL_{nc} = non-carcinogenic safe consumption limit

SCL_{ca} = carcinogenic safe consumption limit

ARL = maximum acceptable individual lifetime risk level (10^{-5}) (USEPA, 2000).

Metal pollution index

The metal pollution index (MPI) was calculated to find and compare the total toxic element pollution in fish's liver and muscle tissues.

$$MPI=(CM_1 + CM_2 + \cdots \ldots \ldots + CM_n)^{1/n} \quad (9)$$

Statistical Analysis

All statistical analyses in this study were performed using the SPSS 25 program. Relationships between fish length-weight, length-metal concentration, and weight-metal concentration were evaluated by using the Pearson correlation test. Paired samples of the T-test and Wilcoxon test were used to compare metal concentrations in fish tissues.

Results and Discussion

Statistical Evaluations Between Heavy Metal Concentrations and Fish Size

The Pearson correlation test was employed to ascertain the relationships between heavy metal concentrations in fish tissues and fish sizes. While significant positive correlations were observed between Pb, Zn and Mn in liver and length-weight ($p<0.05$), no significant correlations were observed between Al, Cd, Cu and Ni and both length and weight ($p > 0.05$). A positive correlation was observed between Fe in the liver and length ($p < 0.05$), but no significant correlation with weight ($p>0.05$). However, no significant correlations were observed between all metals in muscles and length and weight ($p>0.05$). It was observed that there was a positive correlation between the length and weight of the fish ($p<0.01$).

The results demonstrated that only a few metals in the liver exhibited a statistically significant correlation with fish size. This suggests that the relationship between metals accumulated in tissues and fish size is insignificant and disconnected. A similar conclusion was reached in a previous study. (Varol & Kaçar, 2023). However, in contrast to our findings, some studies have observed a negative correlation between metal levels in tissues and fish size, and it has been postulated that metals accumulate at a higher rate in smaller fish. (Ge et al., 2020a; Merciai et al., 2014; Varol et al., 2022).

The Metal Concentration of Fish Tissues and Comparison with Food Safety Guidelines

The accuracy of the proposed method was checked by determining the metal ions using the standard addition method due to the lack of certified reference materials. 0.05 mg/kg and 0.1 mg/kg of standard solutions were added to muscle and liver samples. Validation parameters such as linearity, LOD, LOQ, RSD%, etc., are given in Table 2.

Table 2. The analytical values in FAAS

	Linear regression (y=ax+b)	Correlation coefficients (R^2)	LOD (mg/L)	LOQ (mg/L)	RSD (%)	Recovery (%)
Al	0.0013x – 0.0003	0.9996	0.23	0.77	1.7-7.5	99.6
Cd	0.03x+ 0.0039	0.9995	0.0018	0.006	0.4-4.8	99.9
Cr	0.2125x–0.0038	0.9987	0.015	0.05	0.4-3.6	100.0
Cu	0.2261x+0.0009	0.9997	0.007	0.023	0.2-2.5	97.7
Fe	0.0062x – 0.0005	0.9996	0.24	0.81	0.7-4.8	99.8
Mn	0.0757x+0.0055	0.9984	0.0039	0.013	0.3-4.3	100.0
Ni	0.1137x+0.0005	0.9997	0.001	0.01	0.5-6.2	98.9
Pb	0.0737x–0.0015	0.9992	0.002	0.007	0.3-2.4	102.0
Zn	0.1724x+0.0188	0.9995	0.069	0.23	0.2-1.6	102.2

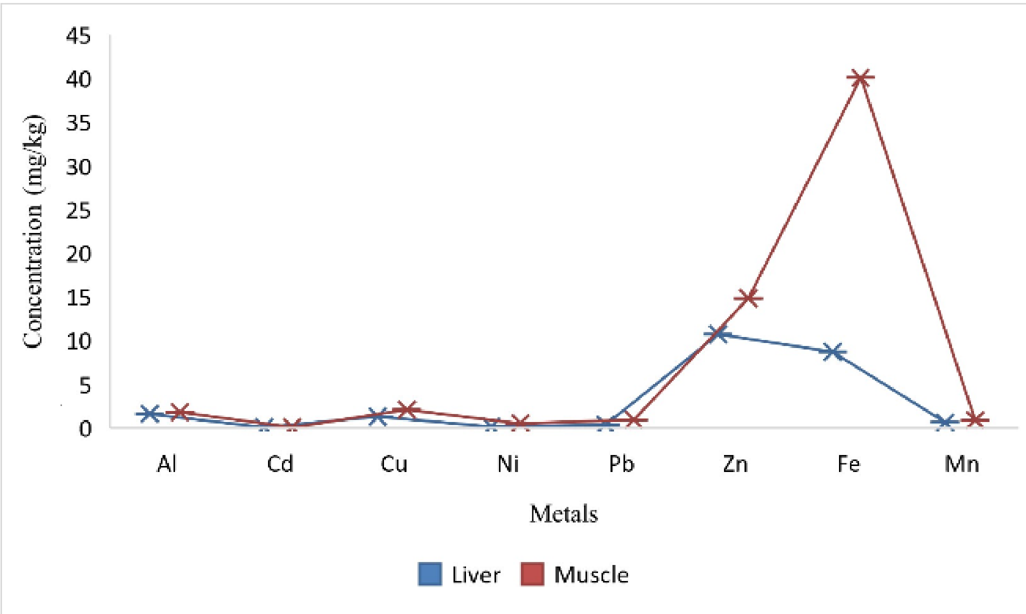

Figure 1. Comparison of metal levels detected in liver and muscle tissues.

Chromium was found below the detection limit in muscle tissues. Among the metals determined in muscle tissue, the highest concentrations are zinc (10.72 $mgkg^{-1}$) and iron (8.69 $mgkg^{-1}$), and the lowest concentrations are cadmium (0.039 $mgkg^{-1}$) and nickel (0.12 $mgkg^{-1}$). Metal concentrations in muscle tissue are in the order Zn>Fe>Al>Cu> Mn>Pb>Ni>Cd.

The chromium concentration in the liver tissues was below the detection limit. Among the metals determined in liver tissue, the highest concentration was observed in iron (40.05 $mgkg^{-1}$), and the lowest was detected in cadmium (0.048 $mgkg^{-1}$). The order of metal concentration determined in liver tissue is as follows: Fe>Zn>Cu>Al>Pb>Mn>Ni>Cd.

When comparing the metal concentrations in the liver and muscles, it was found that all metal concentrations were higher in the liver than in the muscle (Figure 1). Al, Cd and Zn accumulated similarly in both tissues. Cu and Mn accumulated about twice as much in the liver as in muscle. Fe and Ni were significantly higher in the liver than in the muscle. The concentration of Pb in the liver was 2.5 times higher than in the muscle. The liver is metabolically active tissue such as gills, accumulating metals in higher concentrations than other tissues. The liver reflects the fish's past exposure to metals (Ge et al., 2020b; Vetsis et al., 2021; Yilmaz, 2003). On the other hand, muscle tissue has lower metabolic activity than the liver and a longer metal deposition time (Varol et al., 2020; Varol et al., 2022; Vetsis et al., 2021). For this reason, a large amount of metal accumulation is not observed in muscle. Our study confirms that fish tend to accumulate higher levels of metals in their liver. In this respect, our study is sensitive to other studies that compare metal concentrations in various tissues of other fish species (Dirican et al., 2015; Pan et al., 2022; Varol et al., 2022; Vetsis et al., 2021; Yazkan et al., 2002). The average total length-weight and the heavy metal contents detected in the *Trachurus trachurus* fishes are summarised in Table 3.

The contribution of essential elements necessary for human health to the nutritional adequacy of the fish was evaluated by comparing them with the nutrient reference values (NRVs) given in Table 4. The evaluation was limited to muscle tissue samples as humans consume fish muscle tissue. NRVs of Cr, Cu, Fe, Mn, and Zn were 0.04, 1.0, 14.0, 2.0 and 10.0 mg/100g fish, respectively. Since Cr could not be detected in fish muscles, it did not contribute to its nutritional value. The Cu, Fe, Mn, and Zn concentrations in 100 g fish were 0.128, 0.869, 0.059 and 1.072 mg/100 g, respectively. The concentrations of all elements were found to be well below the NRVs. This indicates that the NRV for these elements cannot be achieved even if one portion (200 g) of fish is consumed. This fish species appears to not meet the dietary requirements for the intake of the mentioned elements.

Al, Cd and Pb are non-essential elements. Al is the third most abundant element in the Earth's crust and has no means of biodegradability. Therefore, it readily bioaccumulates in tissues. (Exley & Mold, 2015). This can make Al toxic to aquatic organisms and consumers (Igbokwe et al., 2019). Cd and Pb are known as toxic elements and have no biological function (Genchi et al., 2020). Cd has been proven to be a human carcinogen and has been shown to cause neurodegenerative diseases such as Parkinson's disease (Tamás et al., 2018; Tinkov et al., 2018). It is known that exposure to Pb causes mental disorders and irreversible errors in the mental development of children (O'Connor et al., 2020; Stanaway et al., 2018). The non-essential element concentrations detected in fish muscles were evaluated by comparing them with the maximum permissible limits (ML) values in Table 4. Al (1.59 mg/kg) and Cd (0.039 mg/kg) concentrations were below the reference ML. Pb (0.36 mg/kg) and Mn (0.59 mg/kg) slightly exceeded the reference ML values. The majority of studies conducted with different fish species at different times have shown that consuming fish grown in the Marmara Sea is not risky for health (Cucu et al., 2019; Dökmeci, 2021; Güngör & Kara, 2018). Furthermore, some studies corroborate our findings and suggest that certain metals exceed acceptable limits and should be consumed with care (Köker et al., 2021)

Risk Assessment

Estimated daily intake (EDI)

The EDI represents the estimated amount of metals found in fish that can be consumed daily over a lifetime without posing a health risk. EDI values of all metals were compared with the chronic oral reference doses (RfDo), as shown in Table 4. Fe and Zn stand out in fish muscle tissues with the highest EDI values, whereas Cd, Ni, and Pb have the lowest EDI values (Table 5). EDI values of all metals analysed were much lower than RfDo. These results were correlated with the daily fish consumption in Turkey (18.4 g/day). Consequently, ingesting 18.4 g of these fish daily does not present a health risk associated with the metals. There is one study in the literature that reports results contrary to our study. In this study examining different Trachurus species (*Trachurus mediterraneus*) growing in Marmara, the estimated weekly intake was found to be above the tolerable weekly intake in some regions (Köker et al., 2021).

Target hazard quotient (THQ)

The objective of THQ is to ascertain the non-carcinogenic health risks associated with fish analysed. THQ values of metals determined in fish muscle were evaluated according to whether they were below "1". THQ values of all metals analysed in fish muscle were found below "1". (Table 5.) For this reason, there is no noncarcinogenic health risk for metals in the case of feeding with these fish species grown in the Marmara Sea. Similarly, in a study conducted in 2019, It has been observed that there is no risk in terms of Cd and other metals (except As) in different fish species obtained from the Marmara Sea (Dökmeci et al., 2019).

Target hazard index (THI)

The THI value is employed to ascertain the cumulative non-carcinogenic health risk. The THI value and the percentage of each metal to the THI value (% THI) are presented in Table 5. A THI value exceeding 1 signifies that the ingestion of fish may potentially result in non-carcinogenic health hazards in individuals. The THI value ($1.88x10^{-1}$) in fish was below 1, indicating that a non-carcinogenic health risk does not arise if this fish species from the Sea of Marmara is consumed. Upon examination of the contribution of each metal to the THI value, it becomes evident that the metal with the largest share is Cd (54%), followed by Pb (33.2%). Consequently, it can be posited that Pb and Cd metals represent potential non-carcinogenic risk sources in these fish. Nevertheless, as long as the limits for fish consumption are not exceeded, there is no non-carcinogenic health risk from Pb and Cd metals.

Table 3. Metal concentrations and biological parameters in *Trachurus trachurus*.

	Concentration (mg/kg)			
	Muscle		Liver	
	Min-Max	Mean±SD	Min-Max	Mean±SD
Al	0.73-2.15	1.59±0.38	1.25-2.51	1.76±0.43
Cd	0.022-0.047	0.039±0.0079	0.030-0.064	0.048±0.018
Cr	ND		ND	
Cu	0.89-1.74	1.28±0.29	1.14-3.88	2.05±0.83
Fe	5.61-12.17	8.69±1.76	12.52-105.80	40.05±33.07
Mn	0.39-0.71	0.59±0.094	0.46-1.31	0.86±0.26
Ni	0.14-0.092	0.12±0.018	0.10-0.92	0.51±0.37
Pb	ND±3.56	0.36±1.13	ND±4.92	0.90±1.91
Zn	8.69-13.32	10.72±1.61	10.10-19.11	14.80±3.02
	Properties			
Weight (g)	65.0-170.9	108.0±35.6		
Length (cm)	20.4-27.7	23.3±2.5		

ND: Not detected

Table 4. Maximum permissible limits (ML), nutrient reference values (NRV), chronic oral reference doses (RfDo) and cancer slope factors (CSFo) used for health risk assessment

	ML (mg/kg)	NRV[3] (mg/100g)	RfDo (mg/kg-day)	CSFo[6] $(mg/kg/day)^{-1}$
Al	5.0[1]		1.0E+00[4]	
Cd	0.05[2]		1.0E-04[4]	1.5E+01
Cr	1.0[1]	0.04	3.0E-03[4]	4.2E-01
Cu	4.0[1]	1.0	4.0E-02[4]	
Fe	40.0[1]	14.0	7.0E-01[4]	
Mn	0.55[1]	2.0	1.4E-01[4]	
Ni	0.14[1]		2.0E-02[4]	9.1E-01
Pb	0.3[2]		4.0E-03[5]	8.5E-03
Zn	5.0[1]	10.0	3.0E-01[4]	

[1] (EDQM, 2022); [2] (EU, 2006); [3] (EU, 2011); [4] (USEPA, 2023a); [5] (Nag & Cummins, 2022); [6] (OEHHA, 2023)

Table 5. The results of the calculation of the health risks associated with fish consumption, based on the elemental content of the *Trachurus trachurus*

	Al	Cd	Cu	Fe	Mn	Ni	Pb	Zn
EDI (mg/kg bw/day)	4.17E-04	1.02E-05	3.36E-04	2.28E-03	1.54E-04	3.14E-05	9.36E-05	2.82E-03
THQ	4.17E-04	1.02E-01	8.40E-03	3.26E-03	1.10E-03	1.57E-03	6.24E-02	9.39E-03
THI (%)	0.222	54	4.46	1.73	0.584	0.834	33.2	4.99
LCR		5.08E-05				9.52E-06	2.65E-07	
SCL_{nc} (kg fish/day)	4.41E+01	1.81E-01	2.19E+00	5.64E+00	1.67E+01	1.17E+01	2.95E-01	1.96E+00
SCL_{ca} (kg fish/day)		1.21E-03				6.44E-03	2.31E-01	
THI	1.88E-01							
TCR	6.06E-05							
MPI_{Muscle} (mg/kg fish)	1.42							
MPI_{Liver} (mg/kg fish)	1.58							
MPI_{Total} (mg/kg fish)	3.0							

Lifetime carcinogen risk (LCR) and total cancer risks (TCR)

LCRs were calculated for the metals Cd, Ni and Pb using CSFo values determined by OEHHA. The following criteria were used to conduct the evaluations: if the calculated LCRs fall between 10^{-4} and 10^{-6}, the carcinogenic risk is deemed acceptable. There is no carcinogenic risk if the LCRs are less than 10^{-6}. Conversely, if the LCRs exceed 10^{-4}, the risk is deemed unacceptable (USEPA, 2023). The LCR values calculated for fish are presented in Table 5. The data indicate that the lifetime cancer risk for Cd (5.08×10^{-5}) metal is within acceptable limits when consumed in the indicated quantities. No lifetime cancer risk exists for Ni (9.52×10^{-6}) and Pb (2.65×10^{-7}) metals.

The TCR was calculated to estimate the lifetime probability of developing cancer in individuals exposed to a potential carcinogen through fish consumption. It was evaluated in a manner analogous to that employed for the LCR. The data indicate that the calculated TCR ($6,06 \times 10^{-5}$) is within the minimum acceptable range (10^{-6}-10^{-4}) and does not carry a total cancer risk. Consequently, the evidence indicates that fish do not pose a lifelong cancer risk due to heavy metals.

Safe consumption limits (SCL)

The SCLnc values were calculated separately for each metal to determine the daily quantity of fish a 70-kilogram individual can consume without incurring a non-carcinogenic health risk. Among the metals, Cd (1.81×10^{-1} kg fish/day) and Pb (2.95×10^{-1} kg fish/day) had the lowest SCLnc values, while Al (4.41×10^{1} kg fish/day) had the highest SCLnc value (Table 5). Accordingly, it can be concluded that no health risk is associated with the metals analysed, except for Cd and Pb. However, Cd may pose a non-carcinogenic health risk if more than 181 g of this fish is consumed daily. Similarly, this fish's regular consumption of more than 295 g per day may pose a non-carcinogenic health risk for Pb metal. As the determined consumption values were above the average daily fish consumption in Turkey (18.4 g/day), it was concluded that consuming these fish was not a significant risk.

The SCLca values of the metals Cd, Ni and Pb were calculated to determine the daily amount of fish that a person weighing 70 kg can consume without the risk of developing cancer. The mean SCLca values for Cd, Ni and Pb were 1.21×10^{-3} kg fish/day, 6.44×10^{-3} kg fish/day and 2.31×10^{-1} kg fish/day, respectively (Table 5). For the metals Cd and Ni, the amount of fish that can be consumed without a carcinogenic risk is lower than the average reference consumption of fish (18.4 g/day). Therefore, regular daily consumption of this fish species may be unfavourable regarding Cd and Ni metals. For Pb, the safe consumption limit is higher than the average fish consumption in Turkey. Therefore, carcinogenic health effects are expected if the reference consumption levels are exceeded for Cd and Ni but not for Pb.

Metal pollution index (MPI)

MPI is calculated to assess the environmental pollution level (Bilgin et al., 2023; Fahmy et al., 2023). The degree of contamination can be classified as low, initial, or increasing, depending on the value of MPI. The contamination level is considered low if the MPI is less than one. If MPI equals one, the contamination level is at its initial stage. Finally, if the MPI is greater than one, the contamination level increases (Fahmy et al., 2023). In this study, MPI values in liver and muscle were 1.58 mg/kg fish and 1.42 mg/kg fish, respectively. The concentration of metals in muscle and liver tissues is just above 1. This may indicate that the pollution level of the Marmara Sea is increasing. Furthermore, it has been reported that excessive metal accumulation in the liver compared to muscle indicates water pollution (George, Biju, Martin, & Gerson, 2022). In this study, the quantity of metals accumulated in the liver is less than in the muscle, but they are close to each other. A 2017 study concluded that the water quality of the Marmara Sea does not present a threat to human health or aquatic life (Bozkurt Kopuz & Kara, 2020). A 2020 study proposed that the elevated metal concentrations observed in biota were likely the result of the presence of chemical and environmental wastes in the Marmara Sea (Karabayir, Taskin, Simsek, Aksu, & Caglar, 2020). All these data may indicate that the Marmara Sea is under threat of pollution.

Conclusion

Among the metals examined, Pb and Cd exceeded the tolerable limit, with Pb exceeding the maximum permissible limit. Concentrations of metals were higher in the liver compared to muscle. There was no correlation between metal levels in muscle and fish size, but there was a positive correlation between some metal levels in liver and fish size. The health risk assessment calculations showed that there is no non-carcinogenic health risk associated with the consumption of these fish. The carcinogenic health risk calculations indicated that if the consumption limits were exceeded, there was a Cd and

Ni risk but no lifetime cancer risk. The environmental pollution level of the fish was determined by calculating the metal pollution index, which suggests that the pollution level of the Marmara Sea may be at the initial stage. Consequently, it is recommended that further detailed studies be conducted to control pollution and monitor fish and seafood.

Compliance with Ethical Standards

Conflict of interest: The author(s) declare no actual, potential, or perceived conflict of interest for this article.

Ethics committee approval: This study does not require ethics committee permission or any special permission.

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